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Accumulation of microorganisms on work clothes of workers collecting different types of waste – A feasibility study

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ABSTRACT

Electrostatic dust cloths have previously been used to study microorganisms in settled dust by placing the cloths horizontally on surfaces (called Electrostatic Dust Collectors, EDC). In this study, we investigate whether the same cloths, henceforth called 'E-Cloths', can be used to study accumulation of microorganisms and endotoxin on workers' clothes. This was studied as current methods have limitations. It was examined for waste collection workers, as their work environment is associated with elevated exposure to microorganisms and endotoxin. Each worker received a kit with a T-shirt with an attached E-Cloth on the front, a instruction letter, and a questionnaire. Workers wore the T-shirts during the next two workdays. Unaffected by waste type collected, it was possible to measure the accumulation of bacteria, fungi, and endotoxin from the work environment on the E-Cloths. Geometric mean concentration of 9×10^6 CFU bacteria/m^2, 1×10^7 CFU fungi/m^2, and 4×10^4 endotoxin units/m^2 were found. In total, 100 different bacterial and 25 fungal species were found. The genus *Bacillus* (with 18 species) and *Brevibacterium aurantiacum* were among the dominating bacteria. For fungi, *Penicillium brevicompactum*, *P. commune*, *Penicillium italicum*, and *Aspergillus niger* were most often found. Importantly, mainly environmental bacteria and fungi had accumulated on the E-Cloths and only few skin-related bacterial species were present, showing that accumulation had happened from the work exposure and not workers' skin. In conclusion, the T-shirts with an E-Cloth can be used as a self-administered method for measurement of accumulation of microorganisms and endotoxin from the work environment on waste collection workers' clothes.

1. Introduction

Work with waste collection is associated with elevated exposure to airborne microorganisms including infectious and allergenic species and endotoxin (Krajewski et al., 2002; Madsen et al., 2019a; Madsen et al., 2021). If microorganisms from the work environment accumulate on clothes, it may act as a source of exposure, as it may be transported to the home and cause exposure during, for example, handling of laundry. Therefore, there is an interest in obtaining knowledge about the accumulation of microorganisms on waste collection workers' clothes. Previous studies have mainly focused on health professionals, and contact plates with an agar medium (size: 0.0025 m^2) have been used for taking samples from clothes (Heudorf et al., 2017; Pinon et al., 2013). In a single study, samples were taken from waste workers' clothes and samples were taken using swabs (size: 0.0015 m^2) (Park et al., 2011). In a study with unspecified subjects, both sides of the clothes was vacuumed for 30 s followed by extraction of β-glucan (Siebers et al., 2007). From ancient clothes samples have been taken using adhesive tape (Pangallo et al., 2013). For dust accumulation on work shirts a destructive method with agitation of a piece of the shirt has been used (Cohen and Positano, 1986).

In this study, we investigated whether microorganisms and endotoxin are present in a detectable and quantifiable level on a cloth (size: 0.033 m^2) mounted on T-shirts of waste collection workers. We wanted to study microorganisms accumulating on the cloth rather than on the clothes itself to avoid skin microorganisms from the person wearing the clothes and also to minimize microorganisms and endotoxin deriving from the clothes itself. By using a cloth the sampling process with a swab, vacuum cleaner, tape, or contact plate is avoided. This is of importance because the pressure on the samplers and the structures of the textiles may affect the sampling efficiency (Dolan et al., 2011; Lutz et al., 2013; Moore and Griffith, 2007; Whyte et al., 1989).

We used the type of cloth used in Electrostatic Dust Collectors (EDC) (Noss et al., 2010) which is used for sampling airborne particles when

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placed on a horizontal surface. The method has been used for sampling airborne endotoxin, microorganisms, allergens, and pyrogenic activity in environments with low to high exposure (Frankel et al., 2012; Kilburg-Basnyat et al., 2014; Liebers et al., 2012; Madsen et al., 2019b; Madsen et al., 2012; Normand et al., 2016; Noss et al., 2008; Rocchi et al., 2017; Vijayakumar et al., 2021; White et al., 2019; Zahradnik et al., 2011).

In this study, we investigated whether the cloth used in the EDCs, henceforth called E-Cloth, could be used to study accumulation of microorganisms and endotoxin on waste collection workers' clothes. Thus T-shirts mounted with an E-cloth, an instruction letter, and a short questionnaire on waste type collected were sent to the workers as a kit, in order to test the E-cloth and the feasibility of this kit as an easy way for workers to do a self-administrated assessment.

The following was used to evaluate the successfulness of the E-Cloth as a method to study accumulation of microorganisms and endotoxin on workers' clothes. a) Was it possible for the workers to correctly use the T-shirts and answer a questionnaire. b) Were the E-Cloths still firmly attached to the T-shirts after two days use, and could they easily be remove from the T-shirts post use without damaging them. c) Were concentrations of microorganisms and endotoxin above the detection levels. And d) were the microorganisms on E-Cloths dominated by skin or environmental related microorganisms.

2. Methods

2.1. Study design and the kit

The study took place in the Greater Copenhagen area with one sampling campaign during two whole and consecutive workdays in May 2020, between 6 am and 5 pm with temperatures between 6 and 12 °C. Thirteen male waste collection workers from the same company participated voluntarily. They worked in teams of two, except for one who during one day worked alone. The workers were from 11 different teams, and 2x2 workers were from the same teams. We made a short instruction letter and a questionnaire about types of waste collected, work tasks, and whom they worked with (supplementary file). The questionnaire was pre-tested by the waste workers' trade union for readability. The workers received a kit containing the instruction letter, the questionnaire, and a clean white cotton T-shirt with an E-Cloth. At the end of the day, the workers filled out the questionnaire. The questionnaires showed that, except for one person, each worker acted as both driver of the truck and waste loader (collecting containers with waste and emptying the containers), and that the types of waste collected were: 2xbulky waste (big items not in bags), 2xpaper (not in bags, inside containers), 3xbiowaste (in bags inside containers), 5xresidual waste (in bags inside containers), and 1x3-types container (with 3 separate chambers for plastics, metal (cans etc.), and electronics (nothing in bags)).

2.2. Accumulation on E-Cloths and extraction of microorganisms and endotoxin

The E-Cloth Cloth (ZEEMAN, Alphen, Netherland) was stitched on the T-shirts on a disinfected table using sterile tools and gloves. Each E-Cloth had a surface exposure size of 0.033 m^2 (14.5 × 23 cm). The densities of the E-Cloth and the T-shirts were measured for 4 cloths and 4 pieces of T-shirt cotton (20x20cm). Before weighing, they were equilibrated at constant air temperature and humidity for 24 h. The density of the E-Cloths were 27 g/m^2 and of the T-shirts 164 g/m^2. The T-shirts with the E-Cloths were worn by the waste collection workers throughout the entire workdays. The workers were asked to take off the T-shirts after the first workday and to fold them on the middle, so the two half parts of the E-Cloth covered each other (supplementary file), place them in their personal locker, and wear it again the following morning. After the second workday, they were again asked to take off the T-shirts fold them in the same way and place them in plastic bags, which we collected and brought to the laboratory.

Within 4 h after the completion of the second workday, the E-Cloths were removed from each T-shirt using a seam ripper. Microorganisms and endotoxin were extracted by placing the cloth in 20 mL sterile extraction solution (MilliQ water with 0.85% NaCl and 0.05% Tween80) in 50 mL sterile tubes and shaking at 500 rpm for 30 min at room temperature.

2.3. Cultivation and quantification of bacteria and fungi

For the identification and quantification of cultivable bacteria, suspensions were plated on Nutrient agar (NA; Thermo Fisher Scientific Oxoid, Basingstoke, UK) plates with actidione (cycloheximide; 50 mg/L; Serva, Germany) and on SSI agar (SSI Diagnostica Enteric Medium, SSI Diagnostica, Hillerød, Denmark) plates. NA plates were incubated at 25 °C and 37 °C and were counted after seven and three days, respectively. SSI plates were incubated at 37 °C and colonies were counted after 24 h. For fungi, suspensions were also plated on Dichloran Glycerol agar (DG18; Thermo Fisher Scientific Oxoid, Basingstoke, UK) and on Sabouraud agar (Oxoid, Basingstoke, United Kingdom, with 2 % agar). The DG18 agar plates were incubated at both 25 °C and 37 °C for seven and three days, respectively, and Sabouraud agar plates at 25 °C for 2–3 days. Following incubation, all visible bacterial and fungal isolates were counted for colony forming units (CFU) and identification. The data are expressed CFU/m^2 E-Cloth.

2.4. Identification of bacteria and fungi

Bacterial and fungal isolates were identified by matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) by using a Microflex LT mass spectrometer (Bruker Daltonics, Germany) as described previously (Rasmussen et al., 2021). Spectra were analysed using Bruker Biotyper 3.1 software with the BDAL standard library and filamentous library 1.0. Bacterial isolates were prepared using the extended direct transfer method according to the manufacturer's instructions, while fungal isolates were grown in overnight cultures in Sabouraud Growth Medium (Thermo Fisher Scientific Oxoid) and prepared using the ethanol extraction method.

2.5. Endotoxin and optical density

To determine the concentration of endotoxin, the extracts from the E-Cloths were centrifuged (1000xg) for 15 min. The supernatant was stored at −80 °C until they were analysed (in duplicate) in three dilutions 20, 100, and 200 times using the rFC assay (Lonza Inc.) as described by the supplier and elsewhere (Thorne et al., 2010). The data are expressed as endotoxin units (EU)/m^2 E-Cloth.

To get a measure of how 'dirty' the T-shirts were we determined the Optical Density (OD) of the extraction of the E-Cloths. The absorbance of a sample is measured as a logarithm, and it is directly proportional to the concentration of the light-absorbing material in the sample. The ODs were quantified using the ELx808 Absorbance Microplate reader (BioTekInstruments Inc., Winooski, VT). The dust suspensions were vortexed and an aliquot of 200 mL was added to a transparent microtitre plate and measured at 630 nm (OD_{630}).

All measurements were above the detection limit.

2.6. Data treatment

Concentrations of bacteria were based on the sum of bacteria on $Na_{25°C}$, $NA_{37°C}$, and $SSI_{37°C}$ agar incubated at different temperatures. Fungal concentrations were based the average of fungi on $DG18_{25°C}$ and $Sabauraud_{25°C}$ plus fungi on $DG18_{37°C}$. Based on identifications by MALDI-TOF MS, bacteria were further divided into gram-positive and gram-negative bacteria. Data for bacteria, fungi, and endotoxin were

lognormal distributed and the geometric mean (GM) concentrations (CFU/m^2 cloth) and confidence limits (Cl) were calculated (Limpert et al., 2001). The OD_{630} data were normal distributed. The effect of waste type on concentrations were analysed using Mixed Models with random effect of Team. Pearson's correlation between concentrations of all bacteria, gram-positive bacteria, gram-negative bacteria, all fungi, endotoxin, and OD_{630} were calculated. All statistical analysis were performed in SAS 9.4.

3. Results

3.1. Evaluation of the E-Cloths and a kit for self-administered assessment of microbial accumulation on clothes

Four factors were determined to evaluate the successfulness of the method, and we found that 1) it was possible for the workers to correctly use the T-shirts and answer the questionnaire. The workers informed us that the T-shirts and attached E-Cloths did not disturb their work. All had filled out the questionnaires about waste type collected and numbers of colleagues correctly, and they reported no problems filling in the questionnaire. 2) The E-Cloths were still firmly attached on the T-shirts after the two workdays, and it was possible to remove the cloths from the T-shirts again without contamination and damaging the cloths. 3) Microbial and endotoxin concentrations were above detection levels (Table 1), and 4) E-Cloths were mainly containing environmental bacteria and fungi, with low concentrations of skin related bacteria. Results from points 3) and 4) are described in further detail below.

3.2. Bacteria, fungi, and endotoxin - concentrations

Concentrations of microorganism are presented in Table 1. The concentration of bacteria correlated significantly with the concentration of gram-negative bacteria ($r = 0.75$, $p = 0.0032$) and gram-positive bacteria ($r = 0.96$, $p < 0.0001$), but not with endotoxin, ($r = 0.50$, $p = 0.084$), fungi ($r = -0.41$, $p = 0.16$), and OD_{630} ($r = 0.45$, $p = 0.12$). Gram-negative bacteria correlated significantly with concentrations of endotoxin ($r = 0.71$, $p = 0.0069$) and gram-positive bacteria ($r = 0.85$, $p = 0.0002$). The concentration of fungi tended to correlate negatively with OD_{630} ($r = -0.54$, $P = 0.059$), but not with other examined factors.

The concentration of bacteria ($p = 0.62$), gram-negative bacteria ($p = 0.35$), gram-positive bacteria ($p = 0.27$), endotoxin ($p = 0.18$), and OD_{630} ($p = 0.56$) on the E-Cloths were not affected significantly by the type of waste collected. However, waste type had a significant effect on the concentration of fungi on the E-Cloths ($p = 0.0062$). Thus, it was higher for workers who collected biowaste (GM = $3.1x10^7$ CFU/m^2) or the 3-types of waste ($3.5x10^7$ CFU/m^2) than for workers collecting bulky waste (GM = $1.6x10^6$ CFU/m^2) and paper waste (GM = $8.1x10^5$ CFU/m^2). The concentration of fungi was also higher for workers collecting biowaste than for residual waste ($6.1x10^6$ CFU/m^2), and it was higher for residual waste than paper waste (all ps < 0.0001).

Table 1
Concentrations of bacteria and fungi on E-Cloths (n = 13) as measured under different cultivation conditions, concentration of endotoxin on E-Cloths, and optical density of the suspensions from E-Cloths.

	Average	GM		Confidence limits	Unit
Bacteria					
$NA_{25°C}$	31.9×10^6	3.5×10^6	a	[2.2–5.6 × 10^6]	CFU/m^2
$NA_{37°C}$	131.9×10^5	1.1×10^5	b	[1.0–1.1 × 10^5]	CFU/m^2
$SSI_{37°C}$	5.2×10^4	1.7×10^4	c	[2.6–1.1 × 10^4]	CFU/m^2
Fungi					
$DG18_{25°C}$	13.9×10^6	3.2×10^6	b	[3.0–3.5 × 10^6]	CFU/m^2
$DG18_{37°C}$	19.6×10^3	9.6×10^3	c	[5.6–16.5 × 10^3]	CFU/m^2
$Sabouraud_{25°C}$	14.6×10^6	7.6×10^6	a	[4.3–13.4 × 10^6]	CFU/m^2
Endotoxin	7.9×10^4	4.2×10^4	–	[2.2–8.1 × 10^4]	EU/m^2
OD_{630}	45.8	229	–	[159–299]	OD_{630}/m^2

GM of fungi or bacteria followed by the same letter are not significantly different.

3.3. Bacterial and fungal species

Bacteria and fungi were identified using MALDI-TOF-MS and concentrations are presented in heat maps (Tables 2 and 3). In total, 100 different bacterial species were found and of these 38 species were gram negative. Eighteen different species from the genus *Bacillus* were identified. As an example, 38 different bacterial species were found on E-Cloths of workers collecting biowaste. Some species were only found on a single E-Cloth (Table 2) and some only when using a single cultivation condition (Fig. 1). In total, 61 different species were found on $NA_{25°C}$, 42 species on $NA_{37°C}$ and 22 species on $SSI_{37°C}$.

All together, 25 different fungal species were found and a single species, *Penicillium commune*, was found on all E-Cloths and using both agar media for fungi. Eleven different *Penicillium* species were found after incubation at 25 °C, and one species, *P. citrinum* was also detected at 37 °C (Fig. 1). *Aspergillus fumigatus* was found on 9 of 13 E-Cloths: all 9 samples on $DG18_{37°C}$ and on 3 using $Sab_{25°C}$ but in none using $DG18_{25°C}$. The species *A. niger* was found in 11 of 13 E-Cloths. Of these, in all 11 samples on $DG18_{37°C}$, on 3 using $Sab_{25°C}$, and 3 using $DG18_{25°C}$. Some few fungal species were found only on a single E-Cloth.

4. Discussion

Overall, this study shows that the E-Cloth could stay on the T-shirt for both sampling days, could be removed again, and that the waste collection workers were able to follow our instructions, and that environmental microorganisms accumulated on the E-Cloths on T-shirts to a level, which could be combined with identification and quantification of microorganisms and endotoxin. As described in the introduction, we selected the E-Cloth for this study for several reasons including that it has been well described for sampling sedimenting microbial components and allergens on horizontal surfaces (the EDC-method). The selection of the method with application of a cloth on the T-shirt was chosen as an attempt to minimize contamination by microorganisms from the workers' own skin and from the clothes before work. The microbial identifications revealed many different species and that microorganisms on the E-Cloths were not dominated by skin related species. Bacteria such as *Propionibacterium*, *Acidovorax*, *Pelomonas, Staphylococcus capitis*, *S. cohnii*, *S. epidermidis, S. hominis, S. pasteuri*, *S. pettenkoferi*, *S. simulans*, *S. waneri*, and *S. xylosus* are all commonly present on human skin (Cosseau et al., 2016) and in indoor home dust (Langan et al., 2019; Madsen et al., 2018) – of these only two *Staphylococcus* species were found on the E-cloths. MALDI-TOF MS analysis also revealed that the E-Cloths were dominated by species previously found in the exposure of waste collection, receiving, and sorting workers (Degois et al., 2017; Madsen et al., 2020a; Madsen et al., 2019a; Rasmussen et al., 2021) as e.g.: *Brevibacterium aurantiacum*, *Bacillus cereus*, *B. megaterium, B. pumilus, S. equorum,* all *Penicillium* and *Aspergillus* species and also less frequently found species as e.g. *Corynebacterium callunae, Lelliottia amnigena,* and *Pseudoclavibacter helvolus*. Based on this, we conclude that the E-Cloth method can be used to obtain knowledge about accumulation of microorganisms from the environment on a work shirt.

While the E-cloth has several advantages including a large and fixed sampled area, and a large extracted volume which can be used for several different laboratory analysis, it also has a limitation. Thus, different clothing materials may accumulate microorganisms from the

Table 2
Concentrations (CFU/m^2) of bacterial species on E- cloths on T-shirts of workers (n = 13) collecting bulky, paper, bio, and residual waste, and 3 types of waste (plastic, metal, and electronics). The larger the concentration, the darker the blue color.

Waste		Bulky	Bulky	Paper[1)]	Paper[1)]	Bio[2)]	Bio[3)]	Bio[3)]	Residual	Residual	Residual	Residual	Residual	3-types
CFU/m^2 in total		6.8E+05	5.9E+07	1.8E+08	3.7E+07	2.6E+05	4.7E+05	6.9E+07	4.9E+07	2.9E+05	4.3E+08	2.6E+07	8.7E+07	1.7E+05
Achromobacter mucicolens	N		34290											
Achromobacter sp.	N			1340										
Acinetobacter bohemicus	N			9114833										
Acinetobacter guillouiae	N		34290											
Acinetobacter lwoffii	N	156644	51435					38004				4596	1116	1595
Aerococcus viridans	P			1340	35045967									
Aeromonas veronii	P											4596		
Bacillus altitudinis	P						18921		3475			18383	2734	
Bacillus amyloliquefaciens	P					5126	18921	3131796					1036351	
Bacillus atrophaeus	P						5263		10424			22979	2734	1595
Bacillus cereus	P	2557		19139			2632	11278	7104010			18383	5468	
Bacillus clausii	P									3456				
Bacillus licheniformis	P						5263	3759	10424	3456		32171	2734	3190
Bacillus megaterium	P	7672	8573				18921	6271112	6978925	3456			1036351	
Bacillus mojavensis	P						10526	3759	6949			22979	8202	
Bacillus muralis	P	2557					2632	3759	3475					
Bacillus mycoides	P						2632	3759	10424				2734	3190
Bacillus niacini	P							3759						
Bacillus pumilus	P	10229				5126	7895	3759	20848	6911		45958	21873	
Bacillus simplex	P	10229	25718					3759	3475				2734	1595
Bacillus subtilis	P			19139			34711	11278	62543			68937	32809	
Bacillus thuringiensis	P								10424					
Bacillus vallismortis	P								3475			13787		
Bacillus vietnamensis	P									19875				
Bacillus weihenstephanensis	P	2557								3456				
Brachybacterium conglomeratum	P						18921						1030883	
Brevibacillus borstelensis	P													1595
Brevibacillus parabrevis	P	2557												
Brevibacterium aurantiacum	P						189213			158999	1.1E+08	2.2E+07	5.5E+07	
Brevibacterium sediminis	P									19875				
Brevundimonas diminuta	P		1987											
Chryseobacterium vrystaatense	N			9114833										
Corynebacterium callunae	P			27344498				3759						
Corynebacterium testudinoris	P			9114833										
Curtobacterium flaccumfaciens	P							3131796						
Curtobacterium sp	P							12527186						
Enterobacter cloacae	N			1340								4596		
Enterococcus casseliflavus	P		8407382											
Enterococcus faecium	P							3759						
Erwinia persicina	N										3370085			
Erwinia rhapontici	N										3370085			
Flavobacterium araucananum	N			9114833										
Janthinobacterium lividum	N			9114833										
Kocuria carniphila	P											1845523		
Kocuria polaris	P						18921							
Kocuria rhizophila	P		25718											
Kocuria rosea	P												1586502	
Leclercia adecarboxylata	N	148026												
Lelliottia amnigena	N			9378										
Lysinibacillus boronitolerans	P	2557											2061766	
Lysinibacillus fusiformis	P	7672												
Lysinibacillus sphaericus	P	2557								3456		4596	1030883	
Lysinibacillus xylanilyticus	P								6971975					
Microbacterium aerolatum	P								13943951					

(*continued on next page*)

Table 2 (*continued*)

Microbacterium dextranolyticum	P							12527186						
Microbacterium lacticum	P							3131796						
Microbacterium maritypicum	P							3131796						
Microbacterium phyllosphaerae	P			9114833					6971975					
Microbacterium sp	P							3131796						
Microbacterium testaceum	P							3131796						
Micrococcus luteus	P		176288	19139		47368			1914				7815	4785
Mixta calida	N	148026												
Neomicrococcus lactis	P		33629528											
Ochrobactrum grignonense	N			9116172										
Ochrobactrum tritici	N			1340										
Paenibacillus amylolyticus	P	148026								63080		1845523		
Paenibacillus polymyxa	P									3456				
Pantoea agglomerans	N	2557							3475		20670			
Pantoea calida	N	23962												
Pantoea septica	N									3828				
Paracoccus yeei	N					5126								
Providencia heimbachae	N			1340										
Pseudarthrobacter polychromogenes	P							6263593						
Pseudarthrobacter sulfonivorans	P										3370085			
Pseudoclavibacter helvolus	P			72918660			18921							
Pseudomonas antarctica	N										6740171			
Pseudomonas caricapapayae	N										3370085			
Pseudomonas chlororaphis	N						3828							
Pseudomonas flavescens	N										10254945			
Pseudomonas fluorescens	N										3370085			
Pseudomonas monteilii	N			1340				3828			41340			
Pseudomonas oryzihabitans	N	6061												
Pseudomonas stutzeri	N		1987											
Psychrobacter alimentarius	N										3370085		2061766	
Psychrobacter namhaensis	N												1030883	
Psychrobacter sp	N												3092649	
Rhodococcus erythropolis	P			18229665		15379	37843							
Rhodococcus fascians	P					169173					3370085			
Roseomonas mucosa	N					15379								
Serratia marcescens	N			1340										
Sphingomonas aerolata	N		8407382				18921							
Sphingomonas paucimobilis	N							3131796						
Staphylococcus cohnii	P												2734	
Staphylococcus equorum	P				1708817				6975803		3370085		15151426	
Staphylococcus xylosus	P								7302				2079902	
Stenotrophomonas maltophilia	N								11483				2734	
Stenotrophomonas rhizophila	N						37843	3131796					1030883	
Streptomyces avidinii	P		8407382											
Tsukamurella paurometabola	P							6263593						
Virgibacillus proomii	P													1595

[1)]Colleagues using the same truck (a team); [2)]Only collecting waste – not driving the car; [3)] Colleagues using the same truck. N = gram-negative bacteria, P = gram-positive bacteria.

environment to different degrees. Thus under laboratory conditions *Escherichia coli* adhered to different degrees to different fabrics (Bajpai et al., 2019), and used shirts made of wool or polyester contained more β-glucan than shirts made of cotton (Siebers et al., 2007). Therefor the E-cloth cannot be used to compare different types of work clothes. However, skin bacteria can penetrate woven clothing (Whyte et al., 1978) and may also be sampled. The E-cloth method can be used unaffected by the material of the work clothes and thus to e.g. compare the potential accumulation on clothes in different environments where work clothes of different materials are used and e.g. during different seasons where different types of clothes are used.

The GM concentrations of bacteria ($NA_{25°C}$) and fungi ($DG18_{25°C}$) on

Table 3
Concentrations (CFU/m^2) fungal species on E- cloths on T-shirts of workers (n = 13) collecting bulky, paper, bio, and residual waste, and 3 types of waste (plastic, metal, and electronics). The larger the concentration, the darker the blue color.

Waste	Bulky	Bulky	Paper[1)]	Paper[1)]	Bio[2)]	Bio[3)]	Bio[3)]	Residual	Residual	Residual	Residual	Residual	3-types
CFU/m^2	2.E+06	3.E+06	3.E+06	6.E+05	9.E+07	4.E+07	3.E+07	1.E+07	3.E+06	5.E+06	2.E+07	2.E+07	6.E+07
Aspergillus flavus					1914		2977			3828			
Aspergillus fumigatus		1914	957	957	2884211	2871	5954		26316		40670		1510954
Aspergillus glaucus	17475	34450						1914		3828			
Aspergillus niger	40691		8134		9569	2871	5954	5742	8373	1329187	24402	722488	2999043
Aspergillus oryzae					5742		5954						
Aspergillus versicolor								717703					
Candida famata								717703				6459330	
Cladosporium herbarum		769378	14354	488722					17667				1435407
Cladosporium sp	154613		57416	10253				824030	956938			1435407	4532863
Geotrichum silvicola				623									
Paecilomyces variotii		957						1914					
Penicillium brevicompactum	1014112	120574			1701223	4306220	2614491	79745	743467	130909	2936059	1291866	4381768
Penicillium camemberti	3704	51675		10253	1435407			1568315		32727	2936059		1435407
Penicillium chrysogenum	24883	1214354	14354									1435407	
Penicillium citrinum					1441148							645933	1435407
Penicillium commune	84715	1179904	43062	51888	28601808	4545455	9842789	1488570	53000	98182	2824209	2081340	11785444
Penicillium corylophilum	3704									1381244			
Penicillium digitatum	3704		14354		12280702	3110048	1076555	1621478	37045		1957373	2081340	4532863
Penicillium expansum				623	4572036			2179692	35333	65455			2946361
Penicillium glabrum				12123	1435407	1435407	1076555		17667	32727	978686	1435407	1510954
Penicillium italicum	3704			10876	11749070	3110048	3691046	744285	48252	1381244	978686	2081340	4457316
Penicillium olsonii							1537936						
Penicillium roqueforti						1435407							
Rhizomucor pusillus		957											
Rhizopus oryzae				3349									

[1)]Colleagues using the same truck; [2)]Only collecting waste – not driving the car; [3)] Colleagues using the same truck.

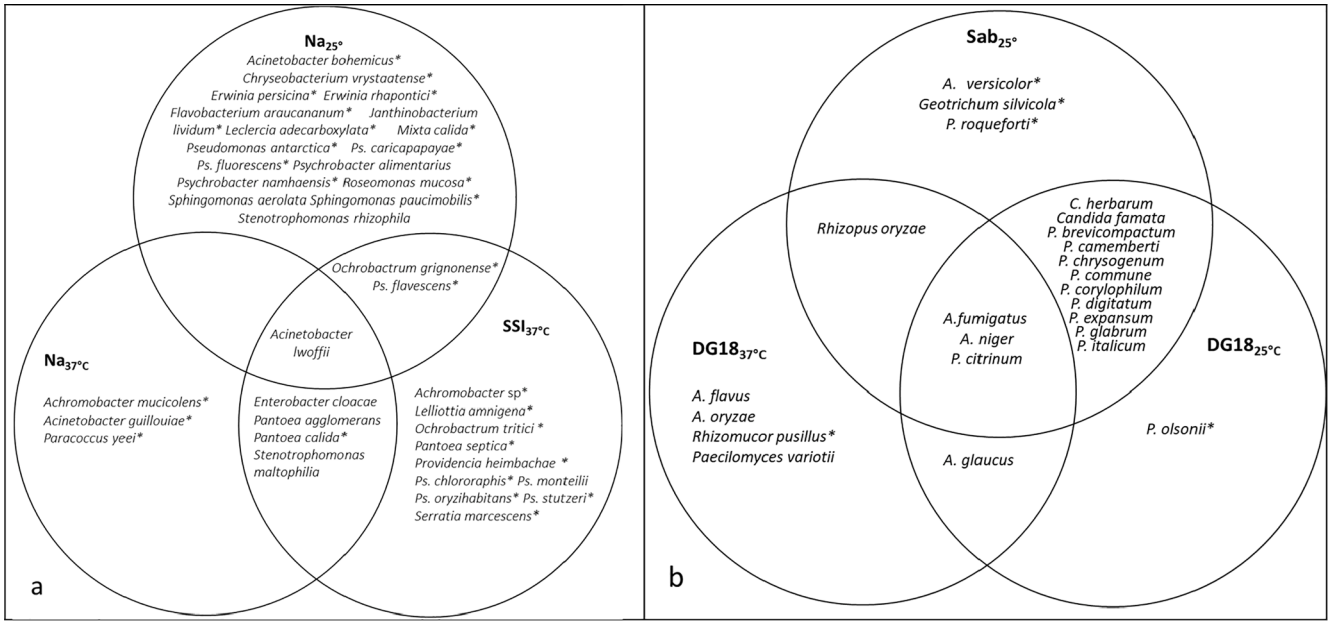

Fig. 1. Venn diagram of species of gram-negative bacteria found on the E-Cloths on T-shirts used for two days as measured using Nutrient agar incubated at 25 °C ($Na_{25°C}$) or 37 °C ($Na_{37°C}$) or SSI agar incubated at 37 °C (**a**) and of fungal species as measured using Sabouraud agar incubated at 25 °C ($Sab_{25°C}$) or DG18 agar incubated at 25 or 37 °C (**b**). *Ps.* = *Pseudomonas, P.* = *Penicillium, A.* = *Aspergillus*, *Species found only in one sample.

the E-Cloth were 3.5×10^6 cfu bacteria/m^2 and 3.2×10^6 cfu fungi/m^2. This method has not been used previously for measuring microorganisms on work clothes, and to get an impression of the measured levels we compare them with studies where the E- cloth has been used for sampling sedimenting microorganism and endotoxin (the EDC-method). Thus, in a study with EDC sampling for 14 days in living rooms concentrations of bacteria and fungi (Madsen et al., 2012) were around 100 times lower than after 2 days use of the T-shirts with E-cloths. The EDC has also been used in control and technical rooms in biowaste receiving plants where the concentrations of bacteria were considerably lower while concentrations of fungi reached the levels found on the E-cloths on the workers T-shirts (Rasmussen et al., 2021). The concentration of endotoxin on the E-Cloths was much higher than concentrations measured for 7 or 14 days sampling in homes using the EDC method (Kilburg-Basnyat et al., 2016; Madsen et al., 2012; Noss et al., 2010). As an expression for the 'dirtiness' of the T-shirts we measured OD_{630}. However, from the present study, we cannot conclude whether OD_{630} is a good estimate of the dustiness of the E-Cloths.

For all laboratory analyses the suspensions extracted from the E-cloths had to be diluted due to the high concentrations of microorganisms and endotoxin. Hence, we expect that the concentrations would also be above the detection level if the workers had only used the T-shirts for 1 day – making it possible to study the accumulation at a daily basis. Another aspect of the high concentration (cfu/m^2) of microorganisms on the E-cloths is that it shows that it would be impossible to use contact plates for measurement of microorganisms on waste collection workers' T-shirts as the concentration (GM) is more than 200 times higher than what can be measured with contact plates.

The E-Cloth has a low density (27 g/m^2) and we expect that this facilitates the extraction in comparison with extraction directly from the T-shirt (density 164 g/m^2) and makes it possible to extract a relatively large area. In a study, authors sampled from workers' gloves and pants by swabbing a 0.0015 m^2 area, and found very high concentrations of bacteria and fungi (Park et al., 2011) even though recovery efficiencies for different swab methods are typically below 5% (Madsen et al., 2020b; Moore and Griffith, 2007; Obee et al., 2007). A study concludes that endotoxin on EDCs is most efficiently extracted with pyrogen-free water with Tween (Noss et al., 2010) with a extraction efficiency for microorganisms on approximately 58% (Madsen et al., 2012). Based on the larger area analysed and the extraction efficiency combined with the high concentrations as well as the species richness measured in the E-Cloths in this study, we expect to obtain a reliable measure for potential accumulation of microorganisms on work clothes using this method. The E-Cloth can be placed on the area(s) of interest on the work clothes and opens up for future studies on microorganisms on work clothes.

As illustrated by the Venn diagram, the three cultivation conditions for bacteria seem all to be necessary to be able to measure the different bacterial species. Some human related bacteria were only found after incubation at 37 °C as e.g. *Enterobacter cloacae* and *Achromobacter mucicolens*. Their absence on $NA_{25°C}$ may be due to competition by faster growing species. Some species as e.g. *Acinetobacter bohemicus* and *Janthinobacterium lividum* were found only at 25 °C, which is in accordance with their inability to grow at 37 °C (Krizova et al., 2014; Valdes et al., 2015). The fungi grew fast on Sabouraud and had to be identified after only 2–3 days. In spite of that, we did not find fewer isolates nor species on Sabouraud than on DG18 agar. However, the DG18 agar plates were easier to handle due to the restricted growth rate. Fungal species found at 25 °C only on DG18 or only on Sabouraud were species found only once, and therefore it is not clear whether the findings are related to the agar medium or the probability of finding a fungus in a sub-sample. Thus, based on this study none of the three cultivation conditions seems redundant.

Increased waste sorting has been implemented as a step towards circular economy (European-Environment-Agency, 2019), however, this is typically associated with reduced waste collection frequency (Madsen et al., 2021), which may furthermore increase exposure to bioaerosols. Taken together, it is therefore even more important to protect workers from exposure at work – but also to reduce so called 'take home exposure'. For a 'take home exposure' perspective, it would be relevant to also analyze the E-Cloths for allergens and antibiotic resistant microorganisms as even low concentrations of these may cause health problems in particular for sensitive people. Some waste types are mainly placed in bags in waste containers, e.g. biowaste, while other waste types, e.g. metal, is often placed directly in waste containers, and e.g. bulky waste may be collected directly by hand. Based on the concentrations of microorganisms and endotoxin found on the E-Cloths of workers collecting bagged waste, the bags do not appear to protect the workers from exposure. It will be relevant to further study how these different waste types, amount of waste collected, and collection methods affect the accumulation of microorganisms on work clothes – especially in light of the concentrations of microorganisms and endotoxin found in this study as well as the presence of Risk group 2 species (Unfallversicherung, 2017): *Enterobacter cloacae, Bacillus cereus*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Aspergillus fumigatus*, and *A. niger*. As examples on occupational health effects *A. fumigatus* has caused respiratory diseases (Hagemeyer et al., 2013), *A. niger* ear infections (Bünger et al., 2000), and *Bacillus cereus* eye infections (Martinez et al., 2007).

In conclusion, by handing out a kit with instructions on the use of the T-shirts with E-Cloths and a questionnaire it was possible for the waste collection workers to use the T-shirts in the correct way and to inform us about the waste type collected. We therefore conclude that the kit can be used for self-administration for assessment of accumulation of microorganisms on work clothes. Unaffected by waste type collected, it was possible to measure the accumulation of bacteria, fungi, and endotoxin from the work environment of waste collection workers using E-Cloths mounted on clean T-shirts. During the two workdays, the E-Cloths seem not to become contaminated by workers' own skin bacteria, and analyses revealed the presence of 100 different cultivable bacterial species and 25 different cultivable fungal species in addition to endotoxin. Due to the high concentrations measured it will also be possible to study the accumulation of microorganisms and endotoxin during only a single workday with waste collection, unlike the two accumulated workdays used in the present study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2021.12.031.

References

Bajpai, S., Bajpai, V., Dey, A., Ghosh, S., Jha, M.K., 2019. Study of adherence kinetics of Escherichia coli on cotton knitted fabrics. Indian Chemical Engineer 61 (3), 296–308.

Bünger, J., Antlauf-Lammers, M., Schulz, T.G., Westphal, G.A., Müller, M.M., Ruhnau, P., Hallier, E., 2000. Health complaints and immunological markers of exposure to bioaerosols among biowaste collectors and compost workers. Occup Environ Med 58, 458–464.

Cohen, B.S., Positano, R., 1986. Resuspension of dust from from work clothings as a source of inhalation exposure. Am Ind Hyg Assoc J 47 (5), 255–258.

Cosseau, C., Romano-Bertrand, S., Duplan, H., Lucas, O., Ingrassia, I., Pigasse, C., Roques, C., Jumas-Bilak, E., 2016. Proteobacteria from the human skin microbiota: species-level diversity and hypotheses. One Health 2, 33–41.

Degois, J., Clerc, F., Simon, X., Bontemps, C., Leblond, P., Duquenne, P., 2017. First metagenomic survey of the microbial diversity in bioaerosols emitted in waste sorting plants. Annals of Work Exposures and Health 61, 1076-1086.

Dolan, A., Bartlett, M., McEntee, B., Creamer, E., Humphreys, H., 2011. Evaluation of different methods to recover meticillin-resistant Staphylococcus aureus from hospital environmental surfaces. J Journal of Hospital Infection 79, 227-230.

European-Environment-Agency, 2019. Waste recycling, in: (Eionet), E.E.I.a.O.N. (Ed.). EEA.

Frankel, M., Timm, M., Hansen, E.W., Madsen, A.M., 2012. Comparison of sampling methods for assessment of indoor microbial exposure. Indoor Air 22, 405–414.

Hagemeyer, O., Bünger, J., van Kampen, V., Raulf-Heimsoth, M., Drath, C., Merget, R., Brüning, T., Broding, H.C., 2013. Occupational allergic respiratory diseases in garbage workers: relevance of molds and Actinomycetes. In: Pokorski, M. (Ed.), Neurobiology of Respiration. Springer, pp. 313–320.

Heudorf, U., Gasteyer, S., Müller, M., Serra, N., Westphal, T., Reinheimer, C., Kempf, V., 2017. Handling of laundry in nursing homes in Frankfurt am Main, Germany, 2016–laundry and professional clothing as potential pathways of bacterial transfer. GMS hygiene infection control 12.

Kilburg-Basnyat, B., Metwali, N., Thorne, P.S., 2014. Effect of deployment time on endotoxin and allergen exposure assessment using electrostatic dust collectors. Annals of Occupational Hygiene 59, 104–115.

Kilburg-Basnyat, B., Peters, T.M., Perry, S.S., Thorne, P.S., 2016. Electrostatic dust collectors compared to inhalable samplers for measuring endotoxin concentrations in farm homes. Indoor air 26 (5), 724–733.

Krajewski, J.A., Tarkowski, S., Cyprowski, M., Szarapinska-Kwaszewska, J., Dudkiewicz, B., 2002. Occupational exposure to organic dust associated with municipal waste collection and management. International journal of occupational medicine and environmental health 15, 289–301.

Krizova, L., Maixnerova, M., Sedo, O., Nemec, A., 2014. Acinetobacter bohemicus sp. nov. widespread in natural soil and water ecosystems in the Czech Republic. Systematic applied microbiology 37 (7), 467–473.

Langan, E.A., Künstner, A., Miodovnik, M., Zillikens, D., Thaçi, D., Baines, J.F., Ibrahim, S.M., Solbach, W., Knobloch, J.K., 2019. Combined culture and metagenomic analyses reveal significant shifts in the composition of the cutaneous microbiome in psoriasis. British Journal of Dermatology 181 (6), 1254–1264.

Liebers, V., van Kampen, V., Bünger, J., Düser, M., Stubel, H., Brüning, T., Raulf-Heimsoth, M., 2012. Assessment of Airborne Exposure to Endotoxin and Pyrogenic Active Dust Using Electrostatic Dustfall Collectors (EDCs). J Toxicol Environ Health A 75 (8-10), 501–507.

Limpert, Eckhard, Stahel, Werner.A., Abbt, Markus, 2001. Log-normal distributions across the sciences: keys and clues. Bioscience 51 (5), 341. https://doi.org/10.1641/0006-3568(2001)051[0341:LNDATS]2.0.CO;2.

Lutz, J.K., Crawford, J., Hoet, A.E., Wilkins, J.R., Lee, J., 2013. Comparative performance of contact plates, electrostatic wipes, swabs and a novel sampling device for the detection of *Staphylococcus aureus* on environmental surfaces. Journal of applied microbiology 115 (1), 171–178.

Madsen, A.M., Frederiksen, M.W., Jacobsen, M.H., Tendal, K., 2020a. Towards a risk evaluation of workers' exposure to handborne and airborne microbial species as exemplified with waste collection workers. Environmental Research 183, 109177. https://doi.org/10.1016/j.envres.2020.109177.

Madsen, A.M., Frederiksen, M.W., Mahmoud Kurdi, I., Sommer, S., Flensmark, E., Tendal, K., 2019a. Expanded cardboard waste sorting and occupational exposure to microbial species. Waste Manag 87, 345–356.

Madsen, A.M., Markouch, A., Frederiksen, M.W., Tendal, K., 2019b. Measurement of dust-borne MRSA in pig farms using different approaches. Journal of applied microbiology 126, 1580–1593.

Madsen, A.M., Matthiesen, C.B., Frederiksen, M.W., Frederiksen, M., Frankel, M., Spilak, M., Gunnarsen, L., Timm, M., 2012. Sampling, extraction and measurement of bacteria, endotoxin, fungi and inflammatory potential of settling indoor dust. Journal of Environmental Monitoring 14 (12), 3230. https://doi.org/10.1039/c2em30699a.

Madsen, A.M., Moslehi-Jenabian, S., Islam, M.Z., Frankel, M., Spilak, M., Frederiksen, M. W., 2018. Concentrations of *Staphylococcus* species in indoor air as associated with other bacteria, season, relative humidity, air change rate, and *S. aureus*-positive occupants. Environ Res 160, 282–291.

Madsen, A.M., Phan, H.U., Laursen, M., White, J.K., Uhrbrand, K., 2020b. Evaluation of methods for sampling of Staphylococcus aureus and other Staphylococcus species from indoor surfaces. Annals of Work Exposures and Health 64, 1020-1034.

Madsen, A.M., Raulf, M., Duquenne, P., Graff, P., Cyprowski, M., Beswick, A., Laitinen, S., Rasmussen, P.U., Hinker, M., Kolk, A., Górny, R.L., Oppliger, A., Crook, B., 2021. Review of biological risks associated with the collection of municipal wastes. Science of The Total Environment, 148287.

Martinez, M.F., Haines, T., Waller, M., Tingey, D., Gomez, W., 2007. Probable occupational endophthalmitis from Bacillus cereus. Archives of environmental occupational health 62 (3), 157–160.

Moore, G., Griffith, C., 2007. Problems associated with traditional hygiene swabbing: the need for in-house standardization. J Journal of applied microbiology 103, 1090-1103.

Normand, A.C., Ranque, S., Cassagne, C., Gaudart, J., Sallah, K., Charpin, D.A., Piarroux, R., 2015. Comparison of air impaction and electrostatic dust collector sampling methods to assess airborne fungal contamination in public buildings. Annals of Occupational Hygiene 60, 161-175.

Noss, I., Doekes, G., Sander, I., Heederik, D.J., Thorne, P.S., Wouters, I.M., 2010. Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. Ann Occup Hyg 54, 651–658.

Noss, I., Wouters, I.M., Visser, M., Heederik, D.J.J., Thorne, P.S., Brunekreef, B., Doekes, G., 2008. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl. Environ Microbiol 74 (18), 5621–5627.

Obee, P., Griffith, C.J., Cooper, R.A., Bennion, N.E., 2007. An evaluation of different methods for the recovery of meticillin-resistant Staphylococcus aureus from environmental surfaces. Journal of Hospital Infection 65 (1), 35–41.

Pangallo, D., Kraková, L., Chovanová, K., Bučková, M., Puškarová, A., Šimonovičová, A., 2013. Disclosing a crypt: microbial diversity and degradation activity of the microflora isolated from funeral clothes of Cardinal Peter Pázmány. Microbiological research 168 (5), 289–299.

Park, D.-U., Ryu, S.-H., Kim, S.-B., Yoon, C.-S., 2011. An assessment of dust, endotoxin, and microorganism exposure during waste collection and sorting. J Air Waste Manag Assoc 61 (4), 461–468.

Pinon, A., Gachet, J., Alexandre, V., Decherf, S., Vialette, M., 2013. Microbiological contamination of bed linen and staff uniforms in a hospital. Advances in Microbiology 2013.

Rasmussen, P.U., Phan, H.U.T., Frederiksen, M.W., Madsen, A.M., 2021. A characterization of bioaerosols in biowaste pretreatment plants in relation to occupational health. Waste Manag 131, 237-248.

Rocchi, S., Valot, B., Reboux, G., Millon, L., 2017. DNA metabarcoding to assess indoor fungal communities: Electrostatic dust collectors and Illumina sequencing. Journal of microbiological methods 139, 107–112.

Siebers, R., Hayes, J., Crane, J., 2007. Upper body clothing is a source of fungal beta-(1,3)-glucan exposure. J Asthma 44, 649–650.

Thorne, P.S., Perry, S.S., Saito, R., O'Shaughnessy, P.T., Mehaffy, J., Metwali, N., Keefe, T., Donham, K.J., Reynolds, S.J., 2010. Evaluation of the Limulus amebocyte lysate and recombinant factor C assays for assessment of airborne endotoxin. Applied environmental microbiology 76 (15), 4988–4995.

Unfallversicherung, I.f.A.d.D.G., 2017. Gestis - Internationale Grenzwerte für chemische Substanzen, http://www. dguv. de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index. jsp.

Valdes, N., Soto, P., Cottet, L., Alarcon, P., Gonzalez, A., Castillo, A., Corsini, G., Tello, M., 2015. Draft genome sequence of Janthinobacterium lividum strain MTR reveals its mechanism of capnophilic behavior. Standards in genomic sciences 10, 1–10.

Vijayakumar, R., Abdulaziz Alfaiz, F., Al-Malki, E.S., Sandle, T., 2021. Assessment of airborne endotoxin in sandstorm dust and indoor environments using a novel passive sampling device in Al Zulfi city, Saudi Arabia-Establishing threshold exposure levels. Saudi Journal of Biological Sciences 28 (2), 1257–1266.

White, J.K., Nielsen, J.L., Madsen, A.M., 2019. Microbial species and biodiversity in settling dust within and between pig farms. Environmental research 171, 558–567.

Whyte, W., Carson, W., Hambraeus, A., 1989. Methods for calculating the efficiency of bacterial surface sampling techniques. Journal of Hospital Infection 13 (1), 33–41.

Whyte, W., Hodgson, R., Bailey, P., Graham, J., 1978. The reduction of bacteria in the operation room through the use of non-woven clothing. Journal of British Surgery 65, 469-474.

Zahradnik, E., Sander, I., Kendzia, B., Fleischer, C., Bruning, T., Raulf-Heimsoth, M., 2011. Passive airborne dust sampling to assess mite antigen exposure in farming environments. J Environ Monit 13, 2638-2644.